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Gliophorus glutinosus sp. nov. (Hygrophoraceae, Agaricales) from Eastern Himalayan region of India

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Abstract

An interesting species of the genus *Gliophorus* (sect. *Glutinosae*), collected from Sikkim Himalaya in India, is described here as *G. glutinosus* **sp. nov.** after thorough morphological examination and phylogenetic analysis. The species is mainly characterised by its strongly glutinous basidiomata throughout, particularly on the twisted stipe, decurrent lamellae with glutinous edge, gelatinised cheilocystidia, presence of pleuropseudocystidia and absence of clamps in hyphae of the pileipellis. This communication includes detailed morphological description, illustrations, comparison with the allied taxa, nrITS based phylogeny of this novel taxon and a key to the species under *Gliophorus* sect. *Glutinosae*.

Keywords

Agaricomycetes, Basidiomycota, *Gliophorus*, Hygrophoraceae, macrofungi, phylogeny, Sikkim, taxonomy

Introduction

Gliophorus Herink (1958: 80) is a member of tribe Humidicuteae, subfamily Hygrocyboideae of Hygrophoraceae (Agaricomycetes, Basidiomycota) and featured by its glutinous basidiomata, variously coloured but never bright red; sinuate or decurrent lamellae, which are sometimes gelatinised at edge; basidiospores smooth, hyaline, thin-walled, inamyloid, ovoid to ellipsoid; presence or absence of ixocheilocystidia; basidia mostly 4-spored, presence of basal clamp in basidia and basidioles; irregular hymenial trama; pileipellis an ixotrichoderm (Lodge et al.

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2014, Singh et al. 2017). This small genus (only 13 members were recorded under this genus in Index Fungorum, http://www.indexfungorum.org/names/Names.asp) encompasses 3 sections: sect. *Gliophorus,* sect. *Glutinosae* (Kühner) Lodge & Padamsee in Lodge et al. (2014: 6) and sect. *Unguinosae* Herink (1959: 81) (Lodge et al. 2014). *Gliophorus* sect. *Glutinosae*, typified by *Gliophorus laetus* (Persoon 1800: 48) Herink (1959: 84), is further characterised by plano-convex pileus that is often indented in centre; green, olive, blue, violet, pink, salmon, yellow, buff, orange or orangish-brown coloured pileus; decurrent lamellae with gelatinised edge; cheilocystidia usually present and embedded in gelatinous matrix (ixocheilocystidia); basidiospores mostly binucleate.

During a macrofungal survey and collection tour to different forested areas of South Sikkim, two of us (DC & KD) came across a very interesting and tiny member of *Gliophorus* sect. *Glutinosae*. After detailed macro- and micromorphological characterisation, coupled with the phylogenetic studies based on the sequence data of nuclear ribosomal internal transcribed spacer (nrITS) region of that species, it was shown to be distinct from any other known species in *Gliophorus* and is proposed here as *G. glutinosus* sp. nov. Detailed morphological description, supporting illustrations and phylogenetic inference is presented here for this novel species.

Material and methods

Morphological study

Macromorphological characters were recorded in the forest and in base-camp from two collections of 13 fresh and dissected young to mature basidiomata. Images of the fresh basidiomata were captured with a Canon Power Shot SX 50 HS. Colour codes and terms are mostly after Methuen Handbook of Colour (Kornerup and Wanscher 1978). Micromorphological characters were observed with a compound microscope (Nikon Eclipse Ni-U). Sections from dried specimens were mounted in a mixture of 5% potassium hydroxide (KOH), 1% Phloxine and 1% Congo red or in distilled water. Micromorphological drawings were prepared with a drawing tube (attached to the Nikon Eclipse Ni) at 1000× magnification. The basidium length excludes sterigmata. Basidiospore measurements were recorded in profile view from 30 basidiospores. Spore measurements and length/width ratios (Q) are recorded here as: minimum–*mean*–maximum. Herbarium codes follow Thiers 2018 (continuously updated).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from dried herbarium specimens (100 mg) using the XcelGen Fungal gDNA Mini Kit (Xcelris Genomics, Ahmedabad, India). The nuclear ribosomal ITS region was amplified using the primers ITS1F and ITS4 (White et al.

1990). Amplification (with PCR) was performed in a 50 µl reaction mix comprising 2 µl template DNA (10–20 ng), 0.5 U Taq DNA polymerase (Sigma-Aldrich, India), 5 µl 10X Taq DNA polymerase buffer, 1 µl 200 µM of each dNTP (Sigma-Aldrich, India), 1 µl 10 pmol primer and the remaining volume made up by H_2O (Sterile Ultra Pure Water, Sigma-Aldrich). This amplification was done using an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) with the following parameters: 5 min step at 95 °C, followed by 30 cycles of 1 min at 95 °C, 30 s at 55 °C and 1 min at 72 °C and a final 7 min extension step at 72 °C. Products from PCR were then purified with QIAquick PCR Purification Kit (QIAGEN, Germany) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The sequencing products were run on 3730×l DNA Analyzer (Applied Biosystems, USA). The raw DNA files were edited and combined using FinchTV and ChromasLite v. 2.01. The sequences generated from two collections (DC 17–28 and DC 17–38) were deposited in GenBank (MH392195 and MH392196).

Phylogenetic analysis

Phylogenetic analyses were based on internal transcribed spacer (ITS) nuclear ribosomal DNA sequences data to establish the phylogenetic placement of the new species. Datasets including reference sequences and outgroup were prepared following relevant literature (Ainsworth et al. 2013, Lodge et al. 2014, Singh et al. 2017), BLAST searches (Altschul et al. 1997) and data retrieved from public databases such as GenBank (Clark et al. 2016) and UNITE (Kõljalg et al. 2013). Multiple sequence alignment was performed using MAFFT v.7 (Katoh and Standley 2013). The aligned loci were loaded in PAUP* 4.0b 10 (Swofford 2001) and the best-fit substitution model of nucleotide evolution (GTR+I+G) was carried out in MrModeltest 3.7 (Posada and Crandall 1998). Bayesian inference was computed in MrBayes v.3.2.2 (Ronquist et al. 2012). Bayesian posterior probabilities (BPP) were calculated in two simultaneous runs with the Markov chain Monte Carlo (MCMC) algorithm (Larget and Simon 1999). Markov chains were run for 1000000 generations, saving a tree every 100th generation. Default settings in MrBayes were used for the incremental heating scheme for the chains (3 heated and 1 cold chain), unconstrained branch length [unconstrained: exponential (10.0)] and uninformative topology (uniform) priors. The analysis was allowed to terminate when the average standard deviation of split frequencies was below 0.01. The first 25% of trees was discarded as burn-in (Hall 2004). Simultaneously, with the same dataset, a full search for the best-scoring Maximum likelihood tree was conducted in RAxML (Stamatakis 2006) using the standard search algorithm (ITS1-5.8S-ITS2 data partitioned, 1000 bootstrap replications). The significant threshold was set above 0.95 for Bayesian posterior probability (BPP) and above 70% for Maximum likelihood bootstrap support (MLB). Phylograms (Figs 1, 2), inferred from Maximum likelihood method and Bayesian phylogeny, are presented showing MLB and BPP values, respectively, for the eligible branches.

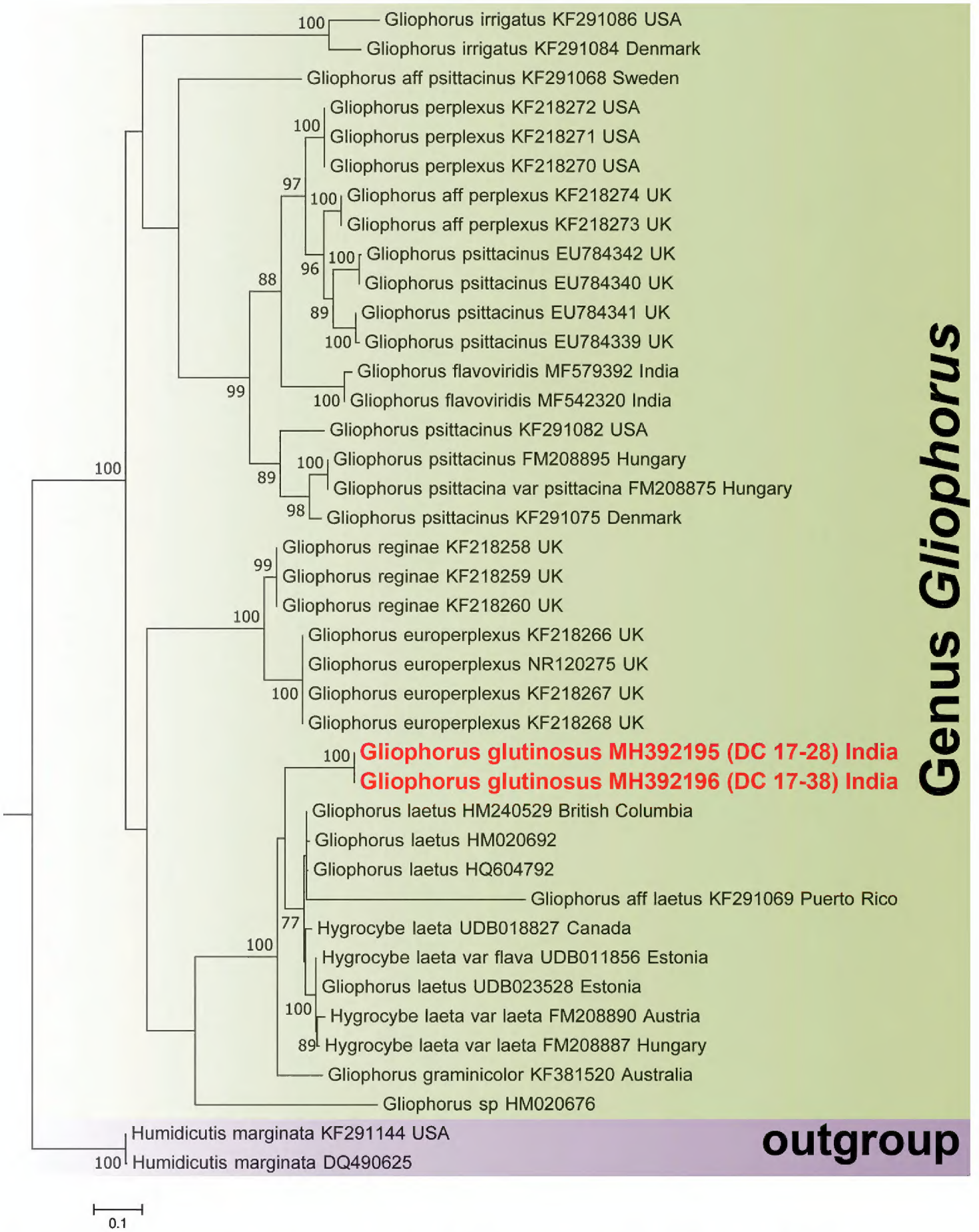
Gliophorus irrigatus KF291086 USA
Gliophorus irrigatus KF291084 Denmark
Gliophorus aff psittacinus KF291068 Sweden
Gliophorus perplexus KF218272 USA
Gliophorus perplexus KF218271 USA
Gliophorus perplexus KF218270 USA
Gliophorus aff perplexus KF218274 UK
Gliophorus aff perplexus KF218273 UK
Gliophorus psittacinus EU784342 UK
Gliophorus psittacinus EU784340 UK
Gliophorus psittacinus EU784341 UK
Gliophorus psittacinus EU784339 UK
Gliophorus flavoviridis MF579392 India
Gliophorus flavoviridis MF542320 India
Gliophorus psittacinus KF291082 USA
Gliophorus psittacinus FM208895 Hungary
Gliophorus psittacina var psittacina FM208875 Hungary
Gliophorus psittacinus KF291075 Denmark
Gliophorus reginae KF218258 UK
Gliophorus reginae KF218259 UK
Gliophorus reginae KF218260 UK
Gliophorus europerplexus KF218266 UK
Gliophorus europerplexus NR120275 UK
Gliophorus europerplexus KF218267 UK
Gliophorus europerplexus KF218268 UK
Gliophorus glutinosus MH392195 (DC 17-28) India
Gliophorus glutinosus MH392196 (DC 17-38) India
Gliophorus laetus HM240529 British Columbia
Gliophorus laetus HM020692
Gliophorus laetus HQ604792
Gliophorus aff laetus KF291069 Puerto Rico
Hygrocybe laeta UDB018827 Canada
Hygrocybe laeta var flava UDB011856 Estonia
Gliophorus laetus UDB023528 Estonia
Hygrocybe laeta var laeta FM208890 Austria
Hygrocybe laeta var laeta FM208887 Hungary
Gliophorus graminicolor KF381520 Australia
Gliophorus sp HM020676
Humidicutis marginata KF291144 USA
Humidicutis marginata DQ490625
Genus Gliophorus
outgroup
0.1

Figure 1. Phylogram resulting from Maximum likelihood analyses of nrITS region. Nodes were annotated with MLB values. MLB values > 70% are shown. Sequences derived from the novel species *Gliophorus glutinosus* (MH392195 and MH392196) are shown in red and bold.

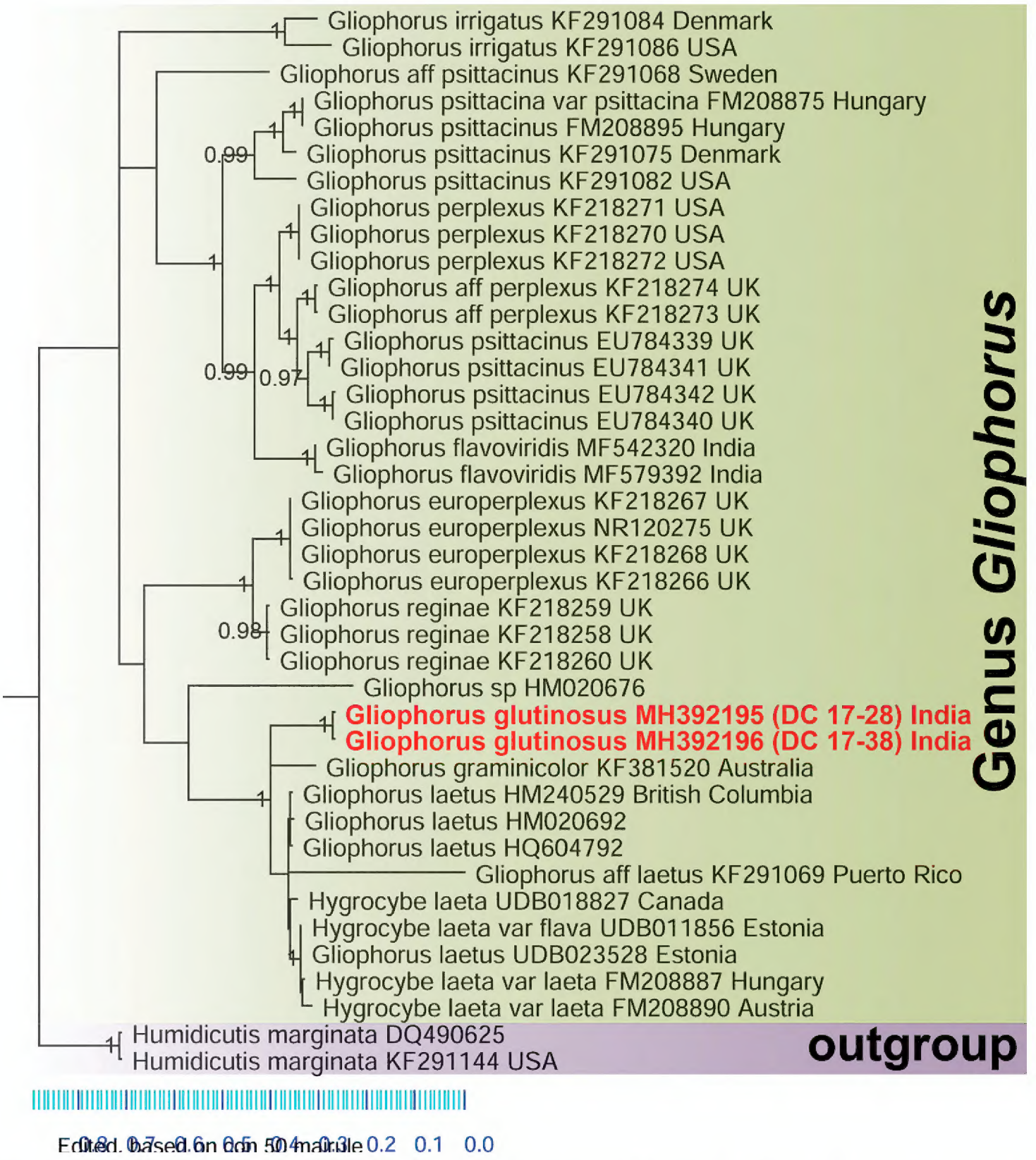

Figure 2. Phylogram resulting from Bayesian phylogeny of nrITS region. Nodes were annotated with BPP values. BPP values > 0.95 are shown. Sequences derived from the novel species *Gliophorus glutinosus* (MH392195 and MH392196) are shown in red and bold.

Results

Phylogeny

The nrITS-sequence dataset consists of 40 sequences. In the Maximum likelihood analysis (Fig. 1), the two Indian collections of the proposed novel species, *Gliophorus glutinosus* (MH392195-DC 17–28 and MH392196-DC 17–38) clustered together (MLB = 100%) as a distinct species and appeared sister to the clade (MLB = 77%) bearing *G. laetus* from Europe, North America, Puerto Rico and Austria (HM240529, HM020692, HQ604792, KF291069, UDB018827, UDB011856, UDB023528, FM208887, FM208890). In turn, *G. laetus* and *G. glutinosus* clustered together as sister (MLB = 100%) to an Australian collection of *G. graminicolor* E. Horak (1973: 176) (KF381520). Similarly, in our Bayesian phylogeny (Fig. 2), the two Indian collections of *Gliophorus glutinosus* clustered together (BPP = 1.00) and appeared sister to the clade bearing *G. laetus* from Europe, North America, Puerto Rico and Austria (HM240529, HM020692, HQ604792, KF291069, UDB018827, UDB011856, UDB023528, FM208887, FM208890). The Australian collection of *G. graminicolor* (KF381520) also appeared as nested (without ancestral information) between the *G. laetus* cluster and *G. glutinosus*.

Taxonomy

***Gliophorus glutinosus* K. Das, D. Chakr. & Vizzini, sp. nov.**
Mycobank: MB 825657
Figs 3, 4

Diagnosis. Distinguished from all the allied taxa by its nrITS sequence and possessing a combination of features like typically twisted stipe submerged under thick gluten, sticky pileus, presence of gluten at lamellar edge, decurrent lamellae, indistinct odour, ixocheilocystidia and presence of pleuropseudocystidia and absence of clamps in pileipellis hyphae.

Type. INDIA. Sikkim: South District, Thangse, 1962 m alt., 27°18.496'N, 88°21.519'E, 23 August 2017, *D. Chakraborty* & *K. Das*, *DC 17–28* (Holotype CAL!).

Etymology. The epithet "*glutinosus*" refers to the highly glutinous stipe surface.

Pileus 5–20 mm diam., convex with a shallow central depression at disc when young, becoming plano-convex at maturity; surface highly glutinous, sticky, sulcate-striate, greyish-orange (6C–B5), brownish-orange (5C5), becoming pale orange to orange white (7C7, 6A3–2) with maturity, sometimes whitish to pastel yellow (2A4) at centre; margin crenate; context ≤ 2 mm thick, concolorous with pileus surface. *Lamellae* subdecurrent to decurrent, moderately close to subdistant (11 per 10 mm at pileus margin), viscid, pale orange to orange white (5A3–2); lamellulae in 3 series; edges glutinous, concolorous with face of lamellae, viscid. *Stipe* 10–60 × 2–5 mm, central,

Figure 3. *Gliophorus glutinosus* (from DC 17–28, holotype). **a–c** Fresh basidiomata in field or in base-camp **d** Transverse section through pileipellis showing ixocutis pattern (under cotton blue) **e, f** Hyphal elements in pileipellis (after removal of gluten) **g** Cheilocystidia (after removal of gluten at lamellae edge) **h** Pleuropseudocystidium **i** Basidioles **j** Hyphae in hymenophoral trama **k** Basidiospores. Scale bars: 20 µm (**d, e, g**); 10 µm (**f, h, i, j, k**).

hollow, cylindrical, often gradually broaden towards base, twisted, longitudinally furrowed, submerged under thick sticky gluten (1 mm); surface upper half pale orange (5A3) and pale yellow to light yellow (4A3–4) towards base. *Taste* and *odour* indistinct. *Spore print* not obtained.

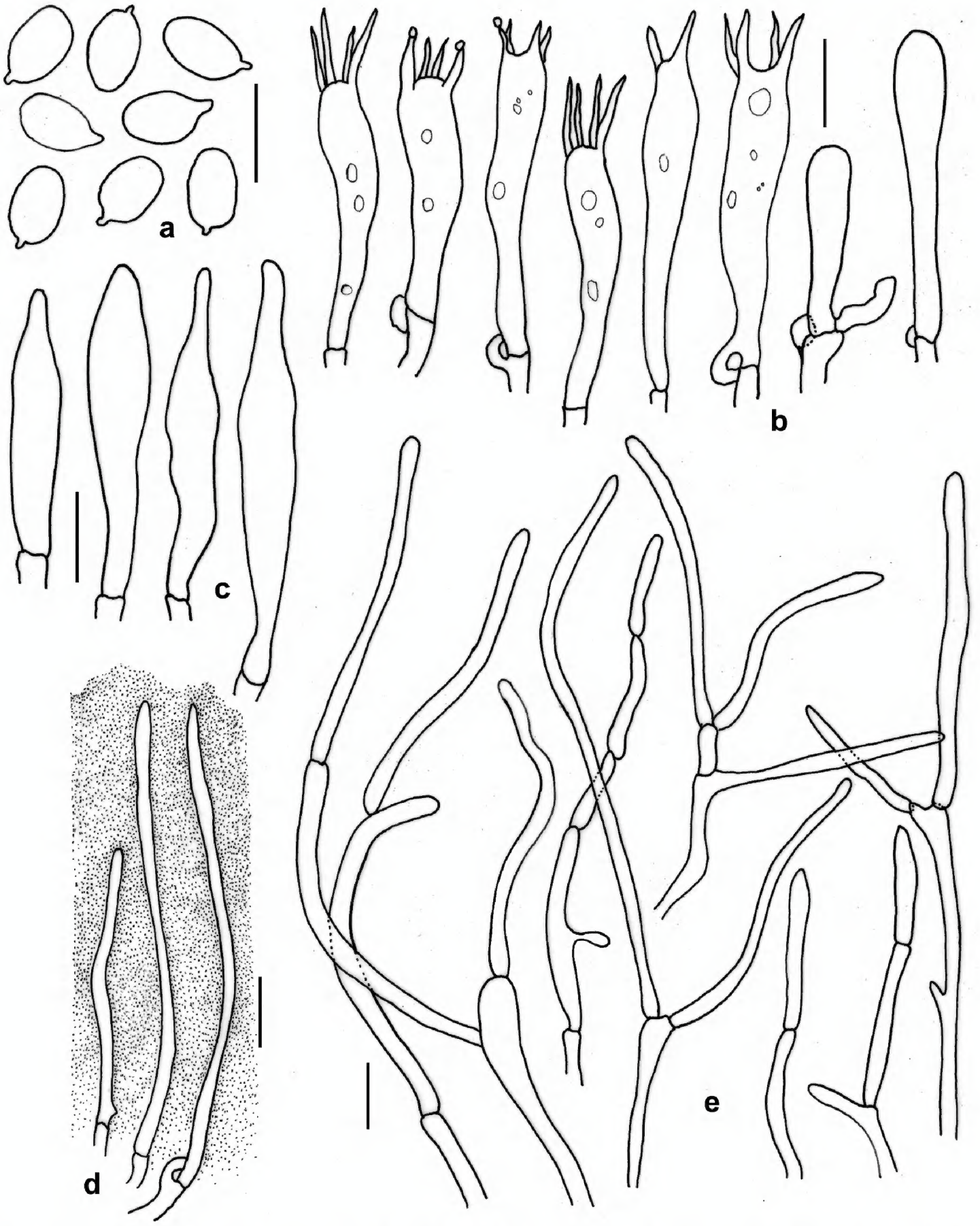

Figure 4. *Gliophorus glutinosus* (from DC 17–28, holotype) **a** Basidiospores **b** Basidia and basidioles **c** Pleuropseudocystidia **d** Ixocheilocystidia **e** Hyphal elements in pileipellis (without gluten and in 5% KOH). Scale bars: 10 µm (**a–e**).

Basidiospores 6–*7*–8 × 3–*4.1*–5 µm (n = 30, Q = 1.5–*1.72*–2.16), elongate-ellipsoid to nearly cylindric, smooth, thin-walled, hyaline, inamyloid, uni- to multiguttulate. *Basidia* 30–38 × 5–7 µm, clavate, thin-walled, with a basal clamp-connection, 2- to 4-spored; sterigmata up to 10 µm long. *Lamellar edge* sterile. *Cheilocystidia* 35–62 × 2–5 µm, slender, occasionally septate, mostly clustered together, gelatinised (embed-

ded in gelatinous matrix). *Pleuropseudocystidia* 31–40 × 5–7 µm, rare, subclavate to appendiculate or fusoid. *Subhymenium* 16–23 µm thick, not gelatinised. *Hymenophoral trama* subregular, consisting of clamped hyphae (3–10 µm diam.), terminal and subterminal cells 17–48 µm long, terminal cells often inflated. *Pileipellis* an ixocutis (when mounted in water or cotton blue), 25–60 µm thick, submerged under thick gluten (seen when mounted with cotton blue), composed of suberect, thin-walled, septate and frequently branched hyphae (observed when mounted in 5% KOH making it free from gluten); terminal elements 15–40 × 2–5 µm, with rounded apex, clamps absent. *Stipitipellis* an ixocutis (when mounted in water or cotton blue) to an ixotrichoderm (when revived in KOH), mostly similar to that of pileipellis.

Habitat/ Distribution. Growing in groups or gregariously on soil amongst leaf-litter of angiospermous plants.

Additional specimen examined. INDIA. Sikkim: South District, Thangse, 1962 m alt., 27°18.496'N, 88°21.519'E, 23 August 2017, *D. Chakraborty* & *K. Das*, DC 17–38 (CAL).

Discussion

The combination of features, such as significantly sticky small basidiomata, distinctively twisted stipe which is completely submerged within a thick (1 mm) transparent layer of gluten, decurrent lamellae with glutinous (sticky) edges, presence of pleuropseudocystidia (sterile elements arising deep in the hymenophoral trama and protruding into the hymenium) and absence of clamps in hyphae of pileipellis, separate *G. glutinosus* from all the known species of *Gliophorus*. Features, such as decurrent lamellae with sticky edges, planoconvex to slightly depressed pileus and presence of ixocheilocystidia, placed the Indian collection under *Gliophorus* sect. *Glutinosae*. In fact, in the phylogenetic analysis (Figs 1–2), the new species forms a strongly supported clade together with *G. laetus*, type species of the sect. *Glutinosae* and with *G. graminicolor*. To our best knowledge, this is the first report of the presence of pleuropseudocystidia in a *Gliophorus* species or, in general, in *Hygrocybe* s.l. (Singer 1986, Boertmann 2010, Lodge et al. 2014). So far, only cheilopseudocystidia have been described as present, albeit rarely, in *Hygrocybe* s.l. (Boertmann 2010, Lodge et al. 2014).

Morphologically, *G. glutinosus* is similar to *G. laetus* [≡*Hygrocybe laeta* (Pers.) P. Kumm. (1871: 112); ≡*Hygrophorus laetus* (Pers.) Fries 1838: 329] but the latter differs by having significantly larger basidiomata (pileus 10–50 mm diam., stipe 30–120 mm long), stipe which is never twisted and less glutinous and showing greyish-lilac tinges at apex; a strongly gelatinised and up to 140 µm thick subhymenium, presence of cuticular clamped hyphae and having an unpleasant odour, described as like burned rubber, burned hair, fish or animal cages (Hesler and Smith 1963, Arnolds 1974, 1990, Boertmann 2010, Bessette et al. 2012). *Gliophorus graminicolor* E. Horak [≡*Hygrocybe graminicolor* (E. Horak) T.W. May & A.E. Wood 1995: 148] from Australia (Tasmania included) and New Zealand is though genetically close to this novel Indian species and can be separated by possessing brown to greenish-brown or grass green coloured

pileus and stipe, less viscid stipe, odour and taste unpleasant, like burnt hair, presence of clamps in pileus hyphae (Horak 1973, 1990, Young and Wood 1997 as *Hygrocybe batesii* A.M. Young (in Young and Wood 1997: 956), Young 1999, 2005, Young and Mills 2002). *Hygrocybe noelokelani* Desjardin & Hemmes (1997: 621), from Hawaii, shows a deep pink, pastel red or pale red pileus, a non-twisted, less viscid stipe, ovoid to broadly ellipsoid, spores (up to 6 µm wide) and presence of large clamp-connections on pileipellis hyphae (Desjardin and Hemmes 1997). *Hygrocybe corallina* Leelav., Manim. & Arnolds (2006: 125), from Kerala, India, has pale red to coral-red basidiomata with bright red lamellae, larger spores [7–10(–11) × 4.5–6.5 µm], clamps observed in all parts of basidioma and the hymenophoral trama regular, made up of medium-sized to long, thin-walled elements, 100–500 × 3–20 µm (Leelavathy et al. 2006).

Key to the species in *Gliophorus* sect. *Glutinosae* worldwide

(* indicated species included in the section are based on morphology alone)

1 Pileipellis as ixocutis.....**2**
– Pileipellis as ixotrichoderm **6**
2 Pileus whitish to pale argillaceous; lamellae adnate.... ***G. pallidus* E. Horak***
– Pileus dark and/or bright coloured; lamellae decurrent **3**
3 Pileus reddish-brown or lilac pink, liver brown; cheilocystidia absent..... ..... ***G. versicolor* E. Horak***
– Pileus greenish-blue, green or orange-brown; cheilocystidia present **4**
4 Pileus orange-brown, strongly glutinous; stipe twisted, embedded in a thick layer of gluten; basidia 2- to 4-spored ..... ***G. glutinosus* (DC 17–28)**
– Pileus green to greenish-blue, surface moderately glutinous; stipe equal; basidia 4-spored.....**5**
5 Pileus green; lamellae whitish with greenish tinge; odour burnt-hair like, unpleasant..... ***G. graminicolor***
– Pileus greenish-blue, turning bluish-lilac with age; lamellae lilac blue or pale greenish-blue; odour none ..... ***G. lilacipes* E. Horak***
6 Pileus pinkish-orange, orange brown or pastel red to pale red; lamellae subdecurrent to decurrent 7
– Pileus green or yellow; lamellae broadly adnate **9**
7 Clamp-connections in pileipellis frequent..... ***G. laetus***
– Clamp-connections in pileipellis very rare.....**8**
8 Pileus orange brown, paler with age; lamellae decurrent, pale yellow; stipe tapering down or broader at middle..... ..... ***H. viscidibrunnea* Bougher & A.M. Young***
– Pileus pink or pastel red or pale red; lamellae subdecurrent, pale pinkish-white; stipe cylindrical .....***H. noelokelani****
9 Pileus greenish; lamellae greenish..... ***G. pseudograminicolor* A.M. Young***
– Pileus yolk yellow to lemon yellow; lamellae yellow ....***G. chromolimoneus* (G. Stev.) E. Horak***

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